August 2012

Assessment of Dim Mutants of the Vibrio harveyi species isolated from the Gulf of Mexico

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ASSESSMENT OF DIM MUTANTS OF THE *VIBRIO HARVEYI* SPECIES ISOLATED FROM THE GULF OF MEXICO

by

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A Thesis Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Master of Science

in Biological Sciences

at

The University of Wisconsin-Milwaukee

August 2012

ABSTRACT

ASSESSMENT OF DIM MUTANTS OF THE *VIBRIO HARVEYI* SPECIES ISOLATED FROM THE GULF OF MEXICO

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The University of Wisconsin-Milwaukee, 2012
Under the Supervision of Dr. Charles Wimpee

Isolating environmental dim and dark mutants of *V. harveyi* gives insight into the positive selection and maintenance of *lux* genes in a natural setting. Mutants were isolated from Boca Ciega Bay, FL., on the basis of the presence of lux genes without visible luminescence when plated on SWC agar. Of 600 isolates, four mutants were found to glow minimally on X-ray film. Of these four, luminescence was rescued in three isolates to a varying degree by addition of aldehyde, a component of the bacterial luminescence reaction. These four *lux* operons were sequenced, and two were shown to have a frameshift in *luxC*, one had a transversion leading to a premature stop codon in *luxA*, while the final strain seemed to be intact. *Lux* operons from 186 and 500 were cloned into *E. coli* to assess luminescence outside of *V. harveyi*'s quorum-sensing circuit and quantitative assays were done to ascertain whether the operon or upstream region were implicated in the reduced luminescence.

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Introduction

Bioluminescence was an early puzzle for biologists. An enzymatic reaction yielding photon emission, bioluminescence is quite a simple system and has evolved 30 or more times in the course of natural history (Hastings, 1983). Bacterial bioluminescence is encoded by the *lux* operon and occurs among many Gram-negative organisms, clustered in γ-Proteobacteria and specifically in the family Vibrionaceae. Further studies utilizing luminescent Vibrios opened the doors to quorum-sensing, a mechanism by which bacterial cells can monitor the presence of small molecules, called auto-inducers, produced by themselves as well as neighboring cells to regulate gene expression in a cell-density dependent manner (Camilli and Bassler, 2006). This phenomenon is widespread across bacteria and its discovery has led to research regarding its role biofilm formation, competence, sporulation, and virulence along with bioluminescence in a broad range of organisms (Davies et al., 1998; Rutherford et al., 2011).

The role of bioluminescence in free-living bacteria has been a frequent debate. DNA repair (Czyz, Plata and Wgrzyn, 2003) and protection from oxidative stress (Szpilewska, Czyz and Wgrzyn, 2003) have been suggested as internal uses for luciferase. In an ecological context, luminescence has been shown to act as a lure for fish, or to more intricately regulate interactions between bacterial cells and potential hosts (Zo et al., 2009, Zarubin et al., 2012).

More interestingly, whole genome sequencing of related Vibrios has yielded insights into genome-wide horizontal gene transfer and mutation rates, suggesting

these organisms exist in a highly plastic and adaptive state (Hunt et al., 2008). In light of this research, assessing environmentally isolated Vibrios and characterizing defects in a strongly retained and phenotypically identifiable set of genes like the *lux* operon allows a glimpse into slight changes, which could lead to shifts in selection and niche space (Hunt et al., 2008). These shifts will possibly become important to understand in the face of climate change, which will potentially change distributions of Vibrio sp. globally, including human and animal pathogens (Oberbeckmann et al., 2012; Vezzulli et al., 2012).

The lux Operon

In terms of the operon itself, bacterial bioluminescence is catalyzed by the enzyme luciferase, which is encoded by *luxA* and *luxB*. Specifically, luciferase facilitates the oxidation of both a reduced flavin mononucleotide ($FMNH_2$) and a long-chain aliphatic aldehyde by molecular oxygen. The aldehyde chains are generated by diverting tetradecanoic acid from the fatty acid biosynthesis pathway, and changing it into a long chain fatty aldehyde (Nealson and Hastings, 1979). This is accomplished using a fatty-acid reductase, which is an enzyme complex encoded by *luxCDE* (Dunlap and Kita-Tsukamoto, 2006; Meighen, 1994).

LuxD cleaves the tetradecanoyl-ACP, the fatty acid is then activated by LuxE with ATP to form fatty acyl-AMP. Finally, LuxC, a NADPH-dependent reductase, reduces activated fatty acyl groups to aldehyde, (Meighen, 1988). Once oxidized, aldehyde returns to a fatty acid state, and is readily converted by the fatty-acid reductase again.

It should also be noted that *Vibrio harveyi* and closely related species have two additional genes, *luxG* and *luxH* (Figure 1). These genes are not necessary for luminescence and have actually been shown to be duplications of genes encoding a flavin reductase (Fre) and RibB, respectively (Nenno and Saigo, 1994; E. Meighen, 1994). Fre and RibB are responsible for generating reduced $FMNH_2$, however no competitive advantage has been seen from having these duplications in the *lux* operon.

Quorum Sensing

To understand bioluminescence in Vibrios and its importance, it is key to discuss quorum-sensing, and cell-to-cell communication in general. Depending on the organism, intercellular and intracellular communication can play a role in group behavior, division of labor, symbiosis, or even spying on other populations of cells (Camilli and Bassler, 2006). The origins of these pathways are ancient, and conserved across species.

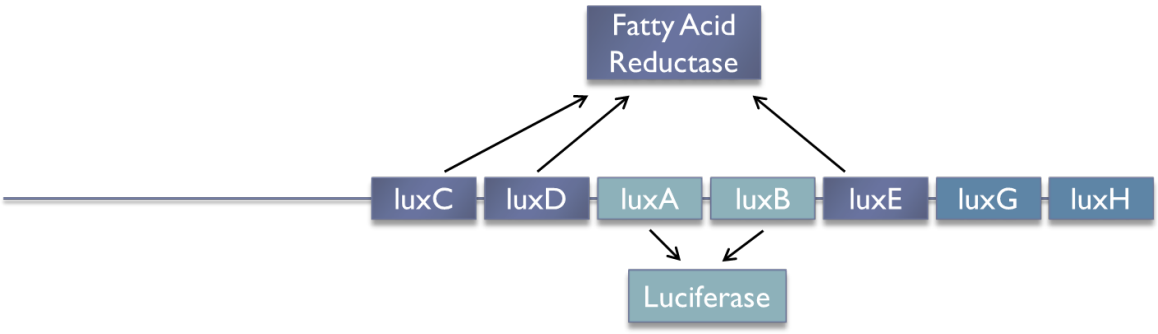

Figure 1. Organization of the *lux* Operon in *Vibrio harveyi.*

To first separate Gram negative and positive species, we find that Gram-positive species frequently use oligopeptides as autoinducers, and quorum-sensing is frequently linked with competence and autolysis. Oligopeptides are not membrane-diffusible and both peptide exporters and two-component systems are necessary to transmit a signal (Camilli and Bassler, 2006). An absence of autoinducers leads to sporulation. This system is very suggestive of a specific lifestyle, one of either an excess of cells and gene-sharing, or dormancy. Gram-negative cells are much more variable. Many Gram-negative species feature an I/R-like system of regulation, made up of a synthase (I) manufacturing a homoserine lactone, which binds to a transcriptional regulator (R). The first I/R system was discovered in *V. fischeri*, LuxI/R. This works in conjunction with another quorum-sensing system, LitR.

Vibrio harveyi Quorum Sensing

In the *Vibrio harveyi* quorum-sensing model, three different small signaling molecules are secreted by bacteria, thereby accumulating in a density-dependent manner. Each of these autoinducers (AI) has a single receptor type, and all three receptors function together to regulate the quorum-sensing pathway (Mok, Wingreen and Bassler, 2003). The first molecule, a homoserine lactone (HAI-1), is responsible for intra-species communication. The second, AI-2, is a furanosyl borate diester and is broadly conserved among the bacterial kingdom, suggesting a role in interspecies communication. The third, CAI-1 is a α-hydroxyketone, was

discovered more recently, and is conserved among related marine microbes. (Higgins et al., 2007, Henke and Bassler, 2004).

Under low cell density conditions, the receptors for these three small molecules act as kinases, which trigger a phosphorylation cascade feeding into the response regulator LuxO, which activates transcription of small inhibitory RNAs (Qrr l-5). These small RNAs destabilize LuxR mRNA when they form complexes with Hfq protein (Figure 2). When the auto inducers are at a higher concentration the receptors change from kinases to phosphatases and the small RNAs are no longer transcribed. LuxR is then free to induce the *lux* operon (Figure 3).

LuxR does not bind to each target gene equally. There are roughly three classes of targets, with high, medium, and low affinity (Pompeani, 2008.) The *lux* operon is a low affinity target, and is only activated when the cell is close to saturated with LuxR. However, the operon has a total of four LuxR binding sites, and it is hypothesized that this results in the late, yet high signal seen during quorum-sensing (van Kessel, personal communication.)

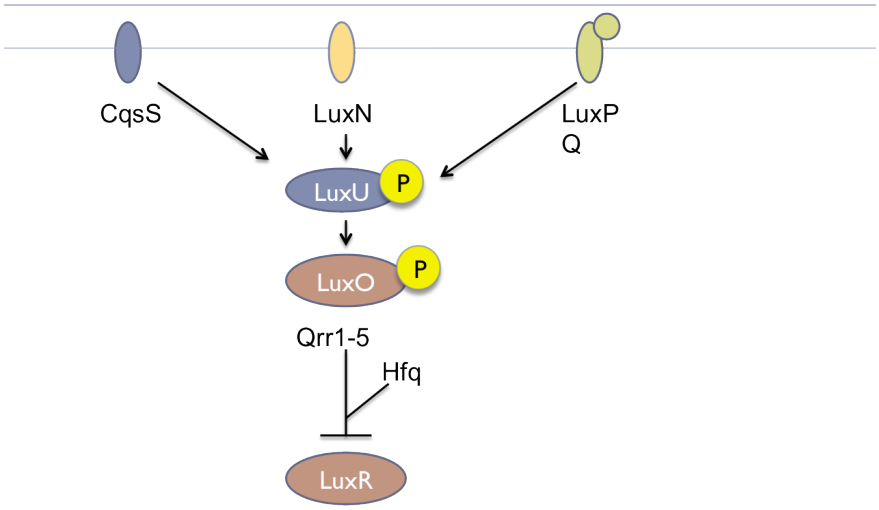

Figure 2. Vibrio harveyi at low cell density. The three hybrid sensor kinases phosphorylate LuxU which activates LuxO, transcribing sRNAs which complex with Hfq to bind *luxR* mRNA.

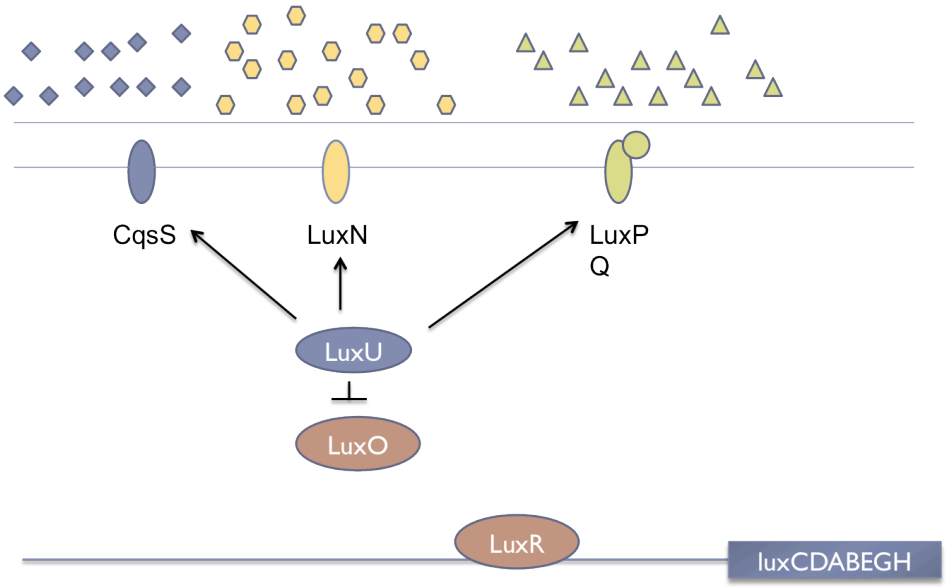

Figure 3. Vibrio harveyi at high cell density. The presence of autoinducers causes the sensor kinases to switch to phosphatase activity, stripping LuxU of activation properties. LuxR is then expressed.

Vibrio Ecology

Vibrios are metabolically diverse and feature high genomic plasticity. *Vibrio* species make up roughly 5% of sampled water communities. Though they are relatively few in number, they are the dominant culturable bacteria in the ocean (Miller et al., 2005). They are metabolically diverse, and are found more frequently attached to surfaces than free-floating in the water column. They also are more frequently found in the coasts, estuaries, and the open ocean (Thompson, Iida and Swings, 2004).

They are generally characterized as chemoorganotrophic mesophiles and facultative fermenters (Thompson, Iida and Swings, 2004). In *Vibrio harveyi*, the 6Mb genome is split between two chromosomes: Chromosome 1, featuring mainly housekeeping genes; and the smaller Chromosome 2, which contains typically niche specific genes (Grimes et al., 2009). Vibrios have exceptionally fast doubling rates, between 12 and 20 minutes in rich media. This can in part be explained by higher gene dosage of replication-associated genes on Chromosome 1 (Dryselius et al., 2008).

Quorum sensing has been shown to play a vital role in the lives of these marine organisms. The oceans are nutrient-poor and not ideal for heterotrophs. Most nutrient accumulations occur on organic and inorganic substrates, and Vibrios can seek out and utilize these nutrients through chemotaxis and developing biofilms (Bassler, 1991; Zobell, 1946). In *Vibrio* species, quorum-sensing activates bioluminescence while down regulating biofilm formation, indicating a strong dispersal phenotype in maturing biofilms (Nadell, 2011). Related to that, it has

been shown that bioluminescence can serve as a lure, known as the Bait Hypothesis, to both small arthropods and fish alike (Zarubin et al., 2012). This further stresses an evolutionary advantage in dispersal, as well as the selective retention and maintenance of *lux* genes in organisms actively expressing them.

The attached lifestyle preferred by these cells, either on surfaces or as aggregates, also makes them prone to high rates of genetic transfer. Most convincingly, chitin-dependent competence has been observed in both *Vibrio cholerae* and *Vibrio vulnificus* in dense, nutrient limited biofilms (Meibom et al., 2005, Gulig et al., 2009). Most Vibrios manufacture several chitinases, either secreted or membrane bound, which are virulence factors in marine arthropod pathogenesis.

In fact, in the presence of such promiscuous genomes, traditional multi-locus analysis may not truly reflect phylogeny; prompting researchers to refer to "ecotypes" rather than species (Keymer and Boehm, 2010; Hunt et al., 2008). Each ecotype may develop a similar gene set, which allows them to most effectively colonize a specific surface, becoming a case of convergent evolution through horizontal gene transfer.

Hypothesis

Members of the Vibrionaeace family have a high frequency of bioluminescence genes compared to other microbes. Selection for these genes is clearly positive in a model of symbiosis, such as *V. fischeri*, however less clear in the open ocean environment. Recent work has suggested a role for luminescence in dispersal, suggesting a possible mode of positive selection and retention of lux genes in Vibrios (Zarubin et al., 2012). Isolation of environmental dim and dark mutants of bioluminescent bacteria gives insight into these processes. To test this (1) seawater was screened for *Vibrio* species which do not visibly glow yet possess the necessary machinery for bioluminescence (*luxCDABE*); (2) operons were cloned and sequenced to assess possible defects in protein-coding genes or the upstream region; and (3) expression of these operons was tested outside of the quorum-sensing context of *Vibrio.*

To address internal lesions, the entire operon of these strains were cloned into a plasmid vector and sequenced. Alignments to known lux sequences determined the presence of deleterious sequences. To address the upstream region, clones were assessed for luminescence outside of the *Vibrio* strains and under different regulation. Elucidating the mechanisms behind the reduced luminescence of these environmentally collected dim isolates yields insight into the regulation and maintenance of lux genes in *Vibrio* sp.

Materials and Methods

Sampling

Seawater samples were collected from Boca Ciega Bay, FL in 2005 and plated on Thiosulfate-citrate-bile salts-sucrose agar (TCBS) agar. Plates were incubated overnight at room temperature. Resultant colonies were replated on Seawater Complete (SWC) agar (3ml of glycerol, 1g of yeast extract, 3g of peptone, and 15g of agar per liter of 75% seawater).

Probing for lux

Isolates were tested for both visual observed luminescence under dark-adapted conditions as well as probed for lux genes. Isolates not exhibiting luminescence but retaining lux genes were further screened for light emission on x-ray film. For x-ray screenings of dim isolates, colonies were streaked onto SWC agar and exposed to x-ray film overnight. Dim isolates were further assessed by addition of tetra-decanal. A single drop of tetra-decanal from a transfer pipet was added to the inside lid of the petri dish and photographs were taken with a 20 minute exposure.

PCR and Sequencing

Primers were made by Integrated DNA Technologies. PCR was performed on a BioRad thermocycler using Gotaq from Promega for short PCR products (<1kb) and Phusion from Finnzymes for long products (>9kb). The following conditions were used in PCR reactions: 5 minute denaturation at 94°c, followed by a 30 cycle sequence of 1 minute at 94°c, 1 minute at 5°c below the lower Tm of the primer pair,

and 30 seconds to 2 minutes at 72°c depending on target length. DNA/plasmid purification was done with GE illustra GFX PCR DNA purification kit, phenol/chloroform extraction or Qiagen Qiaprep columns using manufacturer's protocol, respectively. Sequencing was performed by University of Chicago CRC DNA Sequencing facility. Alignments were performed by Geneious ™ software.

Cloning

Amplified 8kb fragments were obtained from the dim strains using general *V. harveyi* primers flanking the lux operon. These fragments were gel purified and ligated to ampicillin-resistant pGEM-3Zf using an Sph1 site built into the primers. Transformations of the resulting plasmids into XL-10 Gold *E. coli* was done

Strain	Source	Relevant feature
BCB186	This study	Dim
BCB385	This study	Dim
BCB443	This study	Dim
BCB500	This study	Dim
V. harveyi B392	ATCC 33843	Bright
XL-10 Gold *E. coli*	Stratagene	Competent
Top Ten *E. coli*	Invitrogen	Competent
Plasmid	**Source**	**Relevant feature**
pGEM186	This study	Amp-resistant
pGEM385	This study	Amp-resistant
pGEM443	This study	Amp-resistant
pGEM500	This study	Amp-resistant
pGEMB392	This study	Amp-resistant
pLS6Ara-*luxR*	Visick Lab	Arabinose-inducible, Chloremphenicol resistant

Table 1. Names and uses of Strains and plasmids used in study.

according to manufacturer's protocol using 100 micrograms/ml ampicillin to select for the plasmid-carrying isolates on Luria-Bertani (LB) agar.

The transformed cells were then grown overnight in 1.5 ml cultures and the plasmid was extracted using columns. The roughly 11kb plasmids were then inserted into *E. coli* Top Ten cells carrying chloremphenicol-resistant pLS6 featuring an arabinose-inducible *luxR* (*Ara-luxR*). 100 micrograms/ml ampicillin and 25 micrograms/ml chloromphenicol were used to select for both plasmids on LB agar. LuxR induction was done with .2% arabinose.

Light and Turbidity Measurements

The following treatments were used to assess luminesence: *E. coli* + pGEM(dim lux)/pLS6Ara-luxR grown in LB broth with ampicillin, chloramphenicol, and arabinose; pGEMdimlux/pLS6(empty vector) grown in LB broth with ampicillin, chloramphenicol, and arabinose; E. coli + pGEM(dimlux) grown in LB broth with ampicillin; and *V. harveyi* strains grown in SWC broth.

Cultures were grown up overnight from archived samples stored at -80˚ and diluted 1:50 in either SWC or LB broth. Light output was measured by a Lumac/3m Biocounter M2010 luminometer and O.D. was measured at 600 nm on a Versamax Spectrophotometer. Samples were taken every hour over an 8-hour time period.

Results

From the x-ray film and *luxA* screening, four isolates out of 600 were found to have very low but still detectable light output. These strains, named 186, 385, 443, and 500, were further characterized by sequencing their *lux* operons. As mentioned previously, bioluminescence of these strains was only observable on x-ray film. After repeating this experiment, it was noticed that the different strains had different levels of luminescence, although all were too low to be seen by the naked eye (Figure 4.)

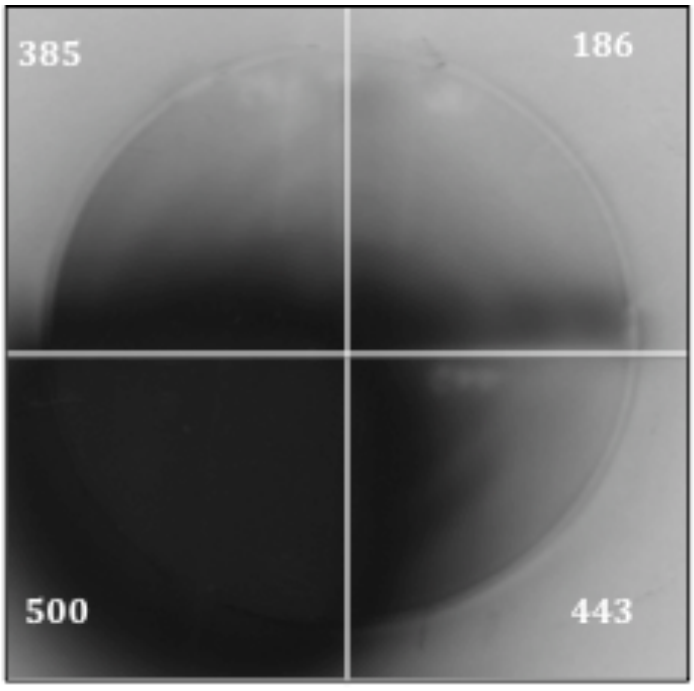

Figure 4. Luminescence of Dim isolates on x-ray film. All four show some signs of functionality. By comparison, B392 (wild type) would turn the film completely black, indicating a high level of luminescence.

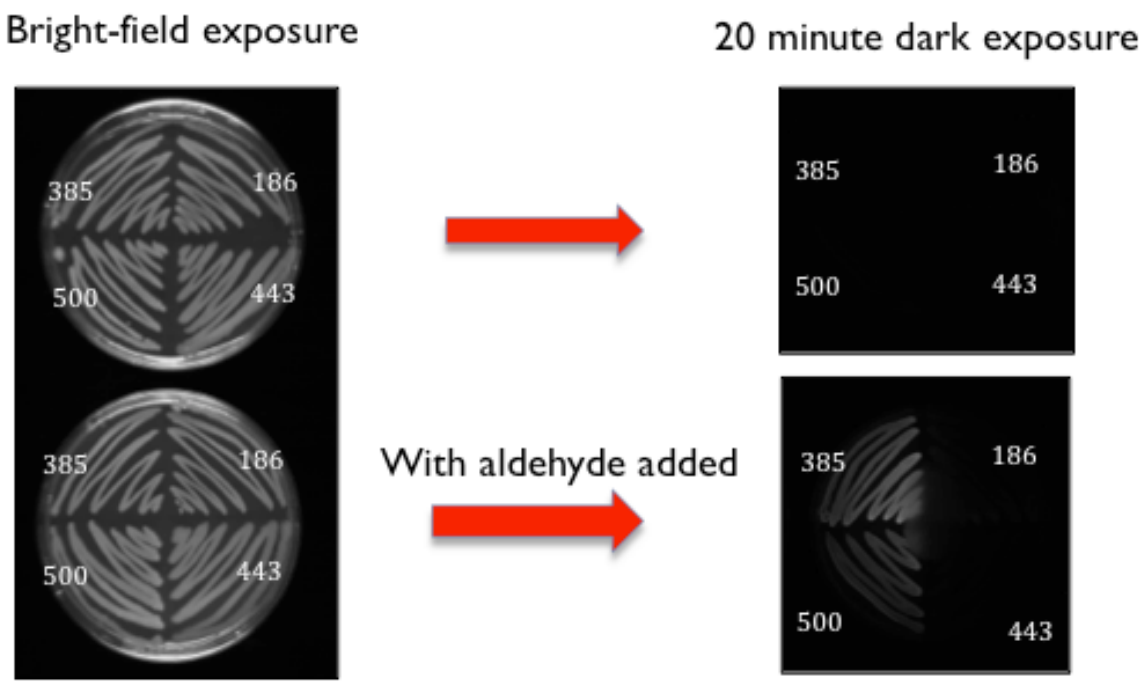

Figure 5. Aldehyde exposure. The four dim strains were exposed to aldehyde, which is one of the reactants in the luciferase reaction. Increased luminescence would suggest that the fatty acid reductase, coded by *luxCDE,* is malfunctioning. Strains 385 and 500 were rescued most strongly. 186 and 443 experienced no notable increase.

Streaked plates of each isolate were then to exposed to aldehyde to ascertain whether any of these strains would benefit from an exogenous source of aldehyde, a method going back many years (Cormier and Strehler, 1953). Luminescence increased significantly in strains 385 and 500, slightly in 186, and no notable improvement in 443 (Figure 5). This indicates that these four strains are potentially ing affected by different mechanisms, and that at least two strains are exhibiting an aldehyde deficiency. Our control strain was not screened on x-ray film, as it would completely expose the film.

Sequencing

BLAST searches identified all four strains as *Vibrio harveyi* through *luxA* sequence analysis. Knowing this, *V. harveyi* specific primers (Appendix A) were used to amplify various *lux* genes in the dim strains. Starting from these fragments, the rest of the operon sequence was obtained through primer-walking. When potential frameshifts were discovered, additional PCR and sequencing was done to verify their authenticity.

Both strains 186 and 500 appear to have frameshifts in *luxC*, (Figure 6b); 186 at position 88 and isolate 500 at position 87 (see also Appendix B). Strain 443 has a transversion resulting in an early stop codon in *luxA* at position 85 (Figure 6c, Appendix C). Strain 385 shows no apparent abnormalities.

Figure 6. Results of sequencing operons of dim *Vibrio harveyi* strains isolated in the Gulf of Mexico. A general map (6a) illustrates where internal lesions (red boxes) were found within the four dim isolates. Sequence alignment of *luxC* show strains 186 and 500 were found to have frameshifts in the front of luxC (6b). Sequence alignment of *luxA* shows strain 443 was shown to have a transversion leading to an early stop codon in luxA (6c).

E. coli Clones

The *lux* operons of dim strains 186 and 500 were cloned into pGEM-3Zf plasmids and inserted into *E. coli* cells featuring an arabinose inducible *luxR* gene. Plate assays confirmed all clones showed arabinose induction of the *lux* operon. Some background was seen in clones featuring p*lux*500 (Figure 7).

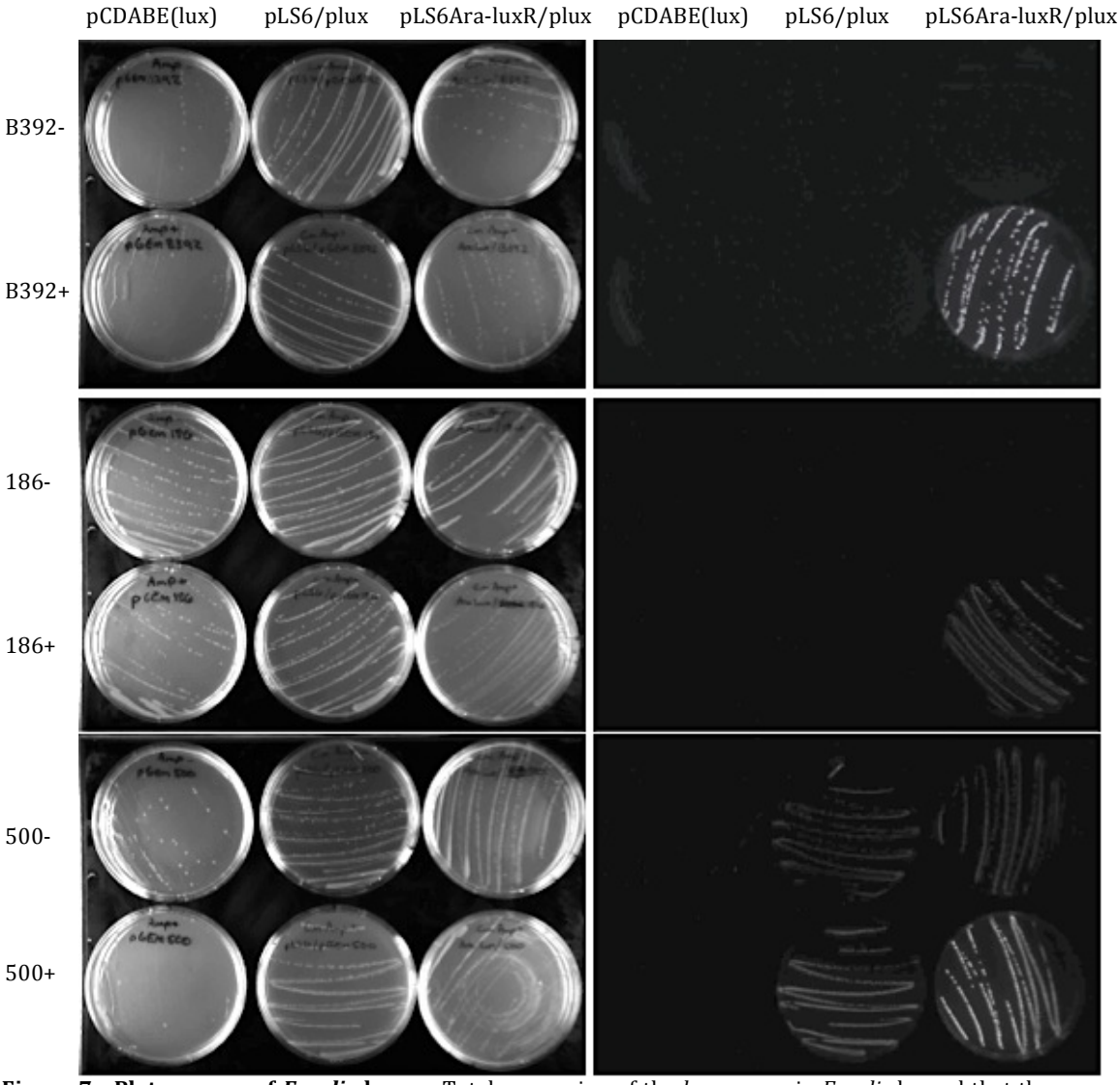

Figure 7. Plate assays of *E. coli* clones. Total expression of the *lux* operon in *E. coli* showed that there was significant induction of *lux* under arabinose. Pictures were taken in bright-field and dark conditions.

Further characterization of 186 and 500 was done using a luminometer coupled with optical density readings to assess luminescence per cell. Growth and luminescence curves were completed for the *Vibrio* strains, as well as *E. coli* clones carrying p*lux*, pLS6/p*lux*, and pLS6-Ara*luxR*/p*lux*. The three *lux* operons were compared by relative luminescence (output/O.D.). In *Vibrio*, the control strain B392 dwarfs light output by the dim isolates, as expected (Figure 8a). In the *E. coli* clones shown below, there is positive induction in the presence of arabinose, though the dim strains do not recover luminescence levels comparable to the output of B392 (Figure 8b).

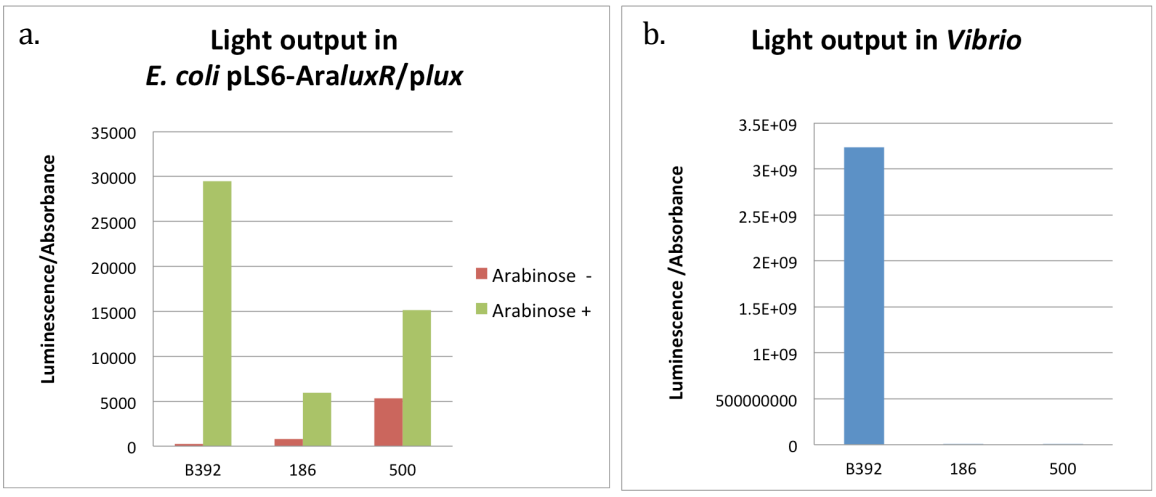

Figure 8. Patterns of light output in A) *Vibrio* isolates and B) *E. coli* clones. Values shown are highest light output/optical density.

Discussion

Sequence Analysis

Sequencing of the four dim operons has yielded some answers. Regarding isolates 186 and 500, the frameshift mutations found in both strains, position 88 and 87 respectively, seems to result in reduced but not complete loss of luminescence. There is a second in-frame start codon at position 127 of *luxC* (see Appendix B), though a previous study has judged that site to be inactive (Miyamoto et al., 1988). The findings of the Miyamoto study, which found no luminescence without the addition of exogenous aldehyde in a clone starting at position 56, between the two start codons, would be at odds with results seen here.

The stop codon in *luxA* found in strain 443 is harder to explain; such a mutation should presumably stop any luminescence. LuxA and LuxB are non-identical subunits of the luciferase enzyme, yet are very similar in structure and possibly arose from a gene duplication event or fragmentation of a monocistronic ancestral gene (Escher, et al. 1989). Previous work in *E. coli* has shown that the individual alpha and beta subunits expressed on plasmids are able to catalyze the luminescence reaction (Waddle 1991). The mutation in isolate 443 has been verified by PCR, and there are no other defects. However, it was the only strain that did not exhibit any aldehyde-related deficiency in plate assays. Therefore luciferase is the limiting factor as predicted, and we can hypothesize that the beta subunit of luciferase still has some functionality.

LuxR Binding affinity

In light of isolate 385's *lux* operon having no apparent lesions, it was of interest to characterize potential binding of LuxR in the upstream region. SmcR is a homolog of LuxR found in *Vibrio vulnificus*. It is virtually identical to LuxR, and comes from the TetR family of repressors that feature a conserved helix-turn-helix binding motif (Pompeani, 2008). The SmcR consensus sequence (Figure 9b) predicts three binding sites upstream of the *lux* operon, and one site within *luxC*; and all four correspond to actual sites shown to be active in LuxR binding assays (van Kessel and Bassler 2012).

a.

Strain	186	385
-289	TTATTTATAGTGTTACAAATAA	TTATTTATAGTGTTACAAAAAA
-153	TTATAGATAAGAAGAACTATAA	TTATAAGTAAATCAAACTATAA
-72	TTTTTAATTAACTTTAAAAAAA	TTATTAATTAACTTTAAAAAAA
+146	TAAATGATCTAAATATTAATAA	TAAATGATCTAAATATAAATAA
Strain	443	500
-289	TTATTTATAGTGTTACAAAGAA	TTATTTATAGTGTTACAAAGAA
-153	TTATAAATAAGGCAAACTATAA	TTATAAATAAGGAAAACTATAA
-72	TTATTAATTAACTTTAAAAAGA	TTATTAATTAACTTTAAAAAAA
+146	TAAATGATCTAAATATAAATAA	TAAATGATCTAAATATAAATAA

b. TTATTGATWWRWTWNTNAATAA

Figure 9. Hypothetical LuxR binding sites. Four sites are predicted upstream of the lux operon, the potentially inactive site is labeled in red (a), based on the SmcR consensus sequence (b).

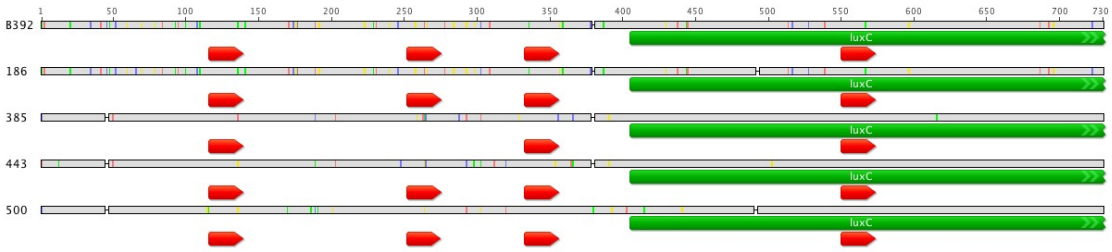

Figure 10. Computational analysis of predicted LuxR Binding. Analysis using SmcR consensus (a) sequence suggests Strain 385 lacks one of the 4 predicted binding sites for LuxR(b). Putative LuxR binding sites indicated by red arrows

Using the Mast program in the online Meme suite (Bailey and Griboskov, 1998), all four binding sites were likely to bind in three of the four isolates (Figure 10). Strain 385, however, appeared to have three LuxR sites (Figure 9a). This may help explain the reduced luminescence in this strain, and its high level of recovery upon addition of aldehyde.

If the fatty acid reductase, a weakly associated complex (Meighen, 1994), were less stable than luciferase, then addition of aldehyde would allow any amount of luciferase present to catalyze the reaction. Further studies will be done to assess its behavior in *E. coli.*

E. coli Clones

In terms of the *E. coli* clones, the results are mixed. As an expression construct, the induction pattern of 186 (luminescence only in the presence of arabinose and LuxR was similar to B392 as predicted since the operons and upstream regions of the two are quite similar. 500 on the other hand exhibited much more leaky expression, glowing even without LuxR (Figure 7).

These results do not explain why 500 was rescued by aldehyde, but 186 was not. This suggests that the frameshift in 500 might somehow not be as deleterious as a very similar mutation in isolate 186. However we may get a clearer picture by uncoupling the upstream regions and operon of these strains and fusing them to B392, including a construct which starts at the putative second start codon.

Conclusion

The work represented here gives unique insight into the multitude (and rarity) of potential mutations that cause Vibrios to lose the ability to bioluminesce. These organisms, cultivated from seawater, represent .01% of the original sampling (O'Grady and Wimpee, 2009). That, coupled with the bait hypothesis, is a strong indication that luminescent Vibrios are more readily distributed than their lesion-carrying counterparts in the water column.

Considering the variety of niche space occupied by these bacteria, one wonders if the same statistic applies to other niches, such as the sediment, surfaces of marine plants and animals, or the gut. The rate of *lux* gene retention may be different for species that prefer a different habitat.

Studies have shown that inheritance of *lux* is almost entirely vertical (Urbanczyk, et al. 2008). This seems curious in light of the high rates of genetic transfer seen among related *Vibrio* species. Possibly there are other characteristics that bioluminescent *Vibrio* share which non-luminescent types do not have, which would potentially keep genetic transfer rates of *lux* low.

However, these isolates were only screened for the presence of *luxA*, which is highly conserved, and in the middle of the *lux* operon. There still lies the possibility of other fragments of *lux* residing in non-luminescent strains. Probing with other *lux* genes may catch other horizontal transfer events, or more dim or dark mutants. In the end, high-throughput sequencing of related *Vibrio* species and mapping recombination events will be able to tell the complete story.

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Appendix

Appendix A: Primers used in sequencing lux operons of dim strains 186, 385, 443, and 500.

Region	Direction	Name	Tm	Sequence
ORF	forward	VHUSORF1	53.4	ACCTGTACCCAAATTGACGC
ORF	forward	VHUSORF2	59.3	GGGGTGGCTTGAGCATAC
Lux C	forward	VHLUXCF	53.7	AACCCAGATGGTGAATCATG
Lux C	forward	VHLUXC F2	60.2	GCAGCGTTGGAAGAGCGAAGA
Lux C	forward	VHLUXC F3	60.9	ATGCAATGCGCCCTCTTCAACG
Lux C	reverse	VHLUXCR	52.1	GGTTTGCTCTATTTCGACAG
Lux D	forward	VHLUXD F1	57	GACTATTGCACACGTGTTGCG
Lux D	forward	VHLUXDF2	56.5	CGTCGACTTTATCGAGCCTGA
Lux D	reverse	VHLUXDR	53.5	CTAAGCCATTTCTGGCGTAC
Lux A	forward	VHLUXA F3	56.8	CACTTATCAGCCACCTGAGCT
Lux A	forward	VHLUXA F4	62.3	GTAGGGACGCCTGAAGAGTGTATCG
Lux A	reverse	VHLUXA R		Ggttttgaagcaaacggttctgaagaa
Lux B	forward	VHLUXB F1	51.7	TCAAAGCGTTCTTCTGATCA
Lux B	forward	VHLUXB F2	58.4	GCCGCGGACCTATTGATGTC
Lux B	reverse	VHLUXB R	53.7	GAGTGGTATTTGACGATGTTGG
Lux E	forward	VHLUXE F1	56.4	ATGGACGTACTTTCAGCGGT
Lux E	forward	VHLUXE F2	56.3	CCACCGTTGAGATCGTTAGAAGA
Lux E	reverse	VHLUXE R	56.2	TCAGTTACCTCCGTCATTCTTAGC
Lux G	forward	VHLUXG F2	55.7	GCCGTTAACTAGCTTCATATTCCG
Lux G	forward	VHLUXG F3	51.2	TCTACTTGTGTGGTCCTTAC
Lux G	reverse	VHLUXG R	56.3	CAGATAAGCGAACGCATCCG
Lux H	forward	VHLUXH F1	53.3	ATGAGCTCAACGTCACTACT
Lux H	forward	LUXH F2	54.4	CGAGGTCATACCGAAGGTAC
Lux H	reverse	VHLUXH R1	56.9	CTAAGACCAACTCACTTCACGCA
MoeB	reverse	MOEB R	59.6	TCCGCTCAATGAGCCTAGCG
ORF	forward	ORF2	45.8	ATTTGAGATCAGTTTTGAAG
ORF	forward	New Orf		ATTTATGCAATATTTATGAG
Lux C	reverse	LuxC R2	59.3	CCCAAGCAACCACTGCATCC
Lux C	reverse	LuxC R3	53.6	TAGTATTGAGGTCACACCGG
Lux C	forward	C3-1	60	GCACTGGGCATTGCAAGCTC
Lux C	forward	C3-2	61	AATCCGTCACCAAAGCCGCC
Lux C	forward	C4	50.4	AAAGAATCGTTGAATCTGGG
Lux C	forward	C5	46.5	GATTTCTTTTTATCCATGGG
Lux A	forward	luxA-1	53.5	GACTCATACGTGAATGCCAC
Lux A	forward	luxA-2	58.2	CGCCTGAAGAGTGTATCGCG
Lux B	forward	luxB-1	50.1	TATTTGGAAGAGTTTGTCCG
Lux B	forward	Dim B2	59.9	ATGGGCGGCTAAGTTAGGGC
Lux E	forward	luxE-1	52.2	ACCATTTGTCTTATCGGTCC
Lux G	forward	luxG-1	50.7	CTGAAATATCAACCTGTCCG
Lux G	reverse	Dim G2R	49.4	ATATGAGGTTAGTTAACGGC
Lux D	forward	443 D4F	55.9	TTGAACAACTCACCATCGCG
Lux A	reverse	443 A5R	51.2	GTACCAAAGACACGGAAATC
ORF	forward	ORF-5-5	45.3	AATAACCCAAGGAATTAATG
ORF	forward	ORF-5-1	44.1	TTTAAAAAAATGATCCAAGG
Lux C	reverse	C5R-1-5	47.2	AATATATGCCCTTCTTCTTG

Appendix B

luxC open reading frame

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                ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
B392            ATGGAAAAAC ACTTACCTTT AATAGTAAAT GGACAAATTA TTTCTACTGA
186             ATGGAAAAAC ACTTACCTTT AATAGTAAAT GGACAAATTA TTTCTACTGA
385             ATGGAAAAAC ACTTACCTTT AATAATAAAT GGGCAAATAG TTTCTACTGA
443             ATGGAAAAAC ACTTACCTTT AATAATAAAT GGGCAAATAG TTTCTACTGA
500             ATGGAAAAAT ACTTACCTTT AATAATAAAT GGGCAGATAG TTTCTACTGA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        60         70         80         90        100
B392            AGAAAATCGA TTTGAGATCA GTTTTGAAGA AAAAAAAGTT AAGATTGATT
186             AGAAAATCGA TTTGAGATCA GTTTTGAAGA AAAAAAA-TT AAGATTGATT
385             AGAAAATCGA TTTGAGATCA GTTTTGAAGA AAAAAAAGTT AAGATTGATT
443             AGAAAATCGA TTTGAGATCA GTTTTGAAGA AAAAAAAGTT AAGATTGGTT
500             AGAAAATCGA TTTGAGATCA GTTTTGAAGA AAAAAA-GTT AAGATTGATT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       110        120        130        140        150
B392            CCTTTAATAA TCTTCATTTA ACCCAGATGG TGAATCATGA TTATTTAAAT
186             CCTTTAATAA TCTTCATTTA ACCCAGATGG TGAATCATGA TTATTTAAAT
385             CCTTTAATCA TGTTCATTTA ACTCAGATGG TGACTCATGA TTATTTAAAT
443             CCTTTAATCA TGTTCATTTA ACTCAGATGG TGACTCATGA TTATTTAAAT
500             CCTTTAATCA TGTTCATTTA ACTCAGATGG TGACTCATGA TTATTTAAAT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       160        170        180        190        200
B392            GATCTAAATA TTAATAACAT CATCAATTTT CTTTATACAA CGGGGCAGCG
186             GATCTAAATA TTAATAACAT CATCAATTTT CTTTATACAA CGGGGCAGCG
385             GATCTAAATA TAAATAACAT CATCAATTTT CTTTATACAA CAGGGCAGCG
443             GATCTAAATA TAAATAACAT CATCAATTTT CTTTATACAA CAGGGCAGCG
500             GATCTAAATA TAAATAACAT CATCAATTTT CTTTATACAA CAGGGCAGCG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       210        220        230        240        250
B392            TTGGAAGAGC GAAGAGTATT CAAGAAGAAG GGCATATATT CGGTCTCTTA
186             TTGGAAGAGC GAAGAGTATT CAAGAAGAAG GGCATATATT CGGTCTCTTA
385             TTGGAAGAGC TAAGAGTATT CAAGAAGAAG GGCATATATT CGGTCTCTTA
443             TTGGAAGAGC GAAGAGTATT CAAGAAGAAG GGCATATATT CGGTCTCTTA
500             TTGGAAGAGC GAAGAGTATT CAAGAAGAAG GGCATATATT CGGTCTCTTA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       260        270        280        290        300
B392            TTACTTATTT GGGGTATTCA CCACAAATGG CAAAACTAGA GGCAAATTGG
186             TTACTTATTT GGGGTATTCA CCACAAATGG CAAAACTAGA GGCAAATTGG
385             TTACTTATTT GGGGTATTCA CCACAAATGG CGAAACTCGA AGCAAATTGG
443             TTACTTATTT GGGGTATTCA CCACAAATGG CGAAACTCGA AGCAAATTGG
500             TTACTTATTT GGGGTATTCA CCACAAATGG CGAAACTCGA AGCAAATTGG
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       310        320        330        340        350
B392            ATTGCAATGA TCCTTTGCTC TAAGAGTGCG CTCTACGACA TTATTGATAC
186             ATTGCAATGA TCCTTTGCTC TAAGAGTGCG CTCTACGACA TTATTGATAC
385             ATTGCAATGA TCCTTTGTTC TAAGAGTGCG CTCTACGACA TTATTGATAC
443             ATTGCAATGA TCCTTTGTTC TAAGAGTGCG CTCTACGACA TTATTGATAC
500             ATTGCAATGA TCCTTTGTTC TAAGAGTGCG CTCTACGACA TTATTGATAC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       360        370        380        390        400
B392            CGAACTTGGC TCTACGCATA TACAAGATGA ATGGCTACCG CAGGGTGAGT
186             CGAACTTGGC TCTACGCATA TACAAGATGA ATGGCTACCG CAGGGTGAGT
385             CGAACTTGGC TCTACGCATA TACAAGATGA ATGGTTACCG CAGGGTGAGT
443             CGAACTCGGC TCTACGCATA TACAAGATGA ATGGTTACCG CAGGGTGAGT
500             CGAACTTGGC TCTACGCATA TACAAGATGA ATGGTTACCG CAGGGTGAGT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       410        420        430        440        450
B392            GTTATGTGAG GGCTTTTCCT AAAGGACGCA CGATGCATTT GCTTGCGGGG
186             GTTATGTGAG GGCTTTTCCT AAAGGACGCA CGATGCATTT GCTTGCGGGG
385             GTTATGTGAG GGCTTTTCCT AAAGGACGCA CGATGCATTT GCTTGCGGGG
443             GTTATGTGAG GGCTTTTCCT AAAGGACGCA CGATGCATTT GCTTGCGGGG
500             GTTATGTGAG GGCTTTTCCT AAAGGACGCA TGATGCATTT GCTTGCGGGG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       460        470        480        490        500
B392            AATGTTCCTC TCTCCGGTGT GACCTCAATA CTACGAGGCA TACTGACGAG
186             AATGTTCCTC TCTCCGGTGT GACCTCAATA CTACGAGGCA TACTGACGAG
385             AACGTTCCTC TCTCCGGTGT GACCTCAATA CTACGAGTCA TACTGACGAG
443             AACGTTCCTC TCTCCGGTGT GACCTCAATA CTACGAGGCA TACTGACGAG
500             AACGTTCCTC TCTCCGGTGT GACCTCAATA CTACGAGGCA TACTGACGAG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       510        520        530        540        550
B392            AAATCAATGT ATTGTGAGAA TGTCAGCATC GGATCCTTTT ACTGCCCACG
186             AAATCAATGT ATTGTGAGAA TGTCAGCATC GGATCCTTTT ACTGCCCACG
385             AAACCAATGT ATTGTGAGAA TGTCAGCATC GGATCCATTT ACTGCCCATG
443             AAACCAATGT ATTGTGAGAA TGTCAGCATC GGATCCATTT ACTGCCCATG
500             AAACCAATGT ATTGTGAGAA TGTCAGCATC GGATCCATTT ACTGCCCATG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       560        570        580        590        600
B392            CGCTAGCGAT GAGCTTTATT GACGTCGATC CGAATCATCC AATTTCTCGT
186             CGCTAGCGAT GAGCTTTATT GACGTCGATC CGAATCATCC AATTTCTCGT
385             CGCTAGCGAT GAGCTTTATT GACGTCGATC CCAACCATCC TATTTCTCGT
443             CGCTAGCGAT GAGCTTTATT GACGTCGATC CCAACCATCC TATTTCTCGT
500             CGCTAGCGAT GAGCTTTATT GACGTCGATC CCAACCATCC TATTTCTCGT
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       610        620        630        640        650
B392            TCCATCTCCG TATTGTATTG GCCTCATGCA TCGGATACGA CACTCGCTGA
186             TCCATCTCCG TATTGTATTG GCCTCATGCA TCGGATACGA CACTCGCTGA
385             TCTATTTCCG TATTGTATTG GCCTCATACA TCGGATACGA CACTCGCTGA
443             TCTATTTCCG TATTGTATTG GCCTCATACA TCGGATACGA CACTCGCTGA
500             TCTATTTCCG TATTGTATTG GCCTCATACA TCGGATACGA CACTCGCTGA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       660        670        680        690        700
B392            AGAGTTACTC AGTCATATGG ATGCAGTGGT TGCTTGGGGG GGGCGGGATG
186             AGAGTTACTC AGTCATATGG ATGCAGTGGT TGCTTGGGGG GGGCGGGATG
385             AGAATTACTG AGTCATATGG ATGCAGTGGT TGCTTGGGGT GGCCGAGATG
443             AGAATTACTG AGTCATATGG ATGCAGTGGT TGCTTGGGGT GGCCGAGATG
500             AGAATTACTG AGTCATATGG ATGCAGTGGT TGCTTGGGGG GGGCGAGATG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       710        720        730        740        750
B392            CCATTGATTG GGCGGTTAAG CATTCTCCTT CACATATCGA TGTTTTGAAG
186             CCATTGATTG GGCGGTTAAG CATTCTCCTT CACATATCGA TGTTTTGAAG
385             CCATTGATTG GGCGGTTAAG CATTCTCCTT CACACATCGA TGTTTTGAAG
443             CCATTGATTG GGCGGTTAAG CATTCTCCTT CACACATCGA TGTTTTGAAG
500             CCATTGATTG GGCGGTTAAG CATTCTCCTT CACACATAGA TGTTTTGAAG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       760        770        780        790        800
B392            TTTGGTCCAA AGAAGAGTTT TACCGTGTTA GACCATCCAG CCGATCTAGA
186             TTTGGTCCAA AGAAGAGTTT TACCGTGTTA GACCATCCAG CCGATCTAGA
385             TTTGGTCCAA AGAAGAGTTT TACCGTGTTA GACCATCTAG CCGATCTAGA
443             TTTGGTCCAA AGAAGAGTTT TACCGTGTTA GACCATCCAG CCGATCTAGA
500             TTTGGTCCAA AGAAGAGTTT TACCGTGTTA GACCATCCAG CCGATCTAGA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       810        820        830        840        850
B392            AGAAGCCGCC TCGGGTGTTG CCCATGATAT TTGCTTTTAT GACCAAAATG
186             AGAAGCCGCC TCGGGTGTTG CCCATGATAT TTGCTTTTAT GACCAAAATG
385             AGAAGCCGCC TCGGGTGTTG CTCATGATAT TTGCTTTTAT GACCAAAATG
443             AGAAGCCGCC TCGGGTGTTG CACATGATAT TTGCTTTTAT GACCAAAATG
500             AGAAGCCGCC TCGGGTGTTG CTCATGATAT TTGCTTTTAT GACCAAAATG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       860        870        880        890        900
B392            CCTGCTTTTC TACTCAGAAT ATTTACTTTT CTGGAGATAA GTATGAAGAA
186             CCTGCTTTTC TACTCAGAAT ATTTATTTTT CTGGAGATAA GTATGAAGAA
385             CCTGCTTTTC TACTCAGAAT ATTTATTTTT CTGGAGATAA GTATGAAGAA
443             CCTGCTTTTC TACTCAGAAT ATTTATTTTT CTGGAGATAA GTATGAAGAA
500             CCTGCTTTTC TACTCAGAAT ATTTATTTTT CTGGAGATAA GTATGAAGAA
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       910        920        930        940        950
B392            TTTAAATTAA AACTTGTTGA AAAACTGAAT CTCTATCAAG AAGTTTTACC
186             TTTAAATTAA AACTTGTTGA AAAACTGAAT CTCTATCAAG AAGTTTTACC
385             TTTAAATCAA AACTTGTTGA AAAACTGAAT CTTTATCAAG AAGTTTTACC
443             TTTAAATCAA AACTTGTTGA AAAACTGAAT CTTTATCAAG AAGTTTTACC
500             TTTAAATCAA AACTTGTTGA AAAACTGAAT CTTTATCAAG AAGTTTTACC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       960        970        980        990       1000
B392            AAAATCAAAA CAAAGTTTTG ATGATGAAGC TTTATTTTCT ATGACTCGTC
186             AAAATCAAAA CAAAGTTTTG ATGATGAAGC TTTATTTTCT ATGACTCGTC
385             AAAATCAAAG CAAAGTTTTG ATGATGAAGC TTTATTTTCT ATGACTCGTC
443             AAAATCAAAG CAAAGTTTTG ATGATGAAGC TTTATTTTCT ATGACTCGTC
500             AAAATCAAAG CAAAGTTTTG ATGATGAAGC TTTATTTTCT ATGACTCGGC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                      1010       1020       1030       1040       1050
B392            TTGAGTGTCA ATTTTCTGGT TTGAAAGTTA TATCAGAACC GGAAAATAAC
186             TTGAGTGTCA ATTTTCTGGT TTGAAAGTTA TATCAGAACC GGAAAATAAC
385             TTGAGTGTCA ATTTTCTGGG TTGAAAGTGA TATCAGAACC GGAAAATAAC
443             TTGAGTGTCA ATTTTCTGGG TTGAAAGTGA TATCAGAACC GGAAAATAAC
500             TTGAGTGTCA GTTTTCTGGG TTGAAAGTTA TATCAGAACC GGAAAATAAC

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                      1060       1070       1080       1090       1100
B392            TGGATGATCA TCGAGTCAGA GCCCGGGGTT GAATATAACC ATCCATTAAG
186             TGGATGATCA TCGAGTCAGA GCCCGGGGTT GAATATAACC ATCCATTAAG
385             TGGATGATCA TCGAGTCAGA GCCCGGGGTT GAATATAACC ATCCATTAAG
443             TGGATGATCA TCGAGTCAGA GCCCGGGGTT GAATATAACC ATCCATTAAG
500             TGGATGATCA TCGAGTCAGA GCCCGGGGTT GAATATAACC ATCCATTAAG

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                      1110       1120       1130       1140       1150
B392            TCGTTGCGTT TATGTCCACA AAATAAATAA GGTTGATGAT GTTGTTCAAT
186             TCGTTGCGTT TATGTCCACA AAATAAATAA GGTTGATGAT GTTGTTCAAT
385             TCGTTGCGTT TATATCCACA AAGTAAATAA GGTTGATGAT GTTGTTCAAT
443             TCGTTGCGTT TATGTCCACA AAGTAAATAA GGTTGATGAT GTTGTTCAAT
500             TCGTTGCGTT TATGTCCACA AAATAAATAA GGTTGATGAT GTTGTTCAAT

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                      1160       1170       1180       1190       1200
B392            ATATAGAAAA ACATCAAACA CAAACGATTT CTTTTTATCC ATGGGAATCT
186             ATATAGAAAA ACATCAAACA CAAACGATTT CTTTTTATCC ATGGGAATCT
385             ATATAGAAAA ACATCAAACA CAAACGATTT CTTTTTATCC ATGGGAATCT
443             ATATAGAAAA ACATCAAACA CAAACGATTT CTTTTTATCC ATGGGAATTT
500             ATATAGAGAA ACATCAAACA CAAACGATTT CTTTTTATCC ATGGGAATCT
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       1210       1220       1230       1240       1250
B392            TCCAAGAAAT ATCGAGATGC ATTCGCCGCA AAAGGGGTAG AAAGAATCGT
186             TCCAAGAAAT ATCGAGATGC ATTCGCCGCA AAAGGGGTAG AAAGAATCGT
385             TCCAAGAAAT ATCGAGATGC ATTCGCCGCA AAAGGAGTAG AAAGAATCGT
443             TCCAAGAAAT ATCGAGATGC ATTCGCCGCA AAAGGAGTAG AAAGAATCGT
500             TCCAAGAAAT ATCGAGATGC ATTCGCCGCA AAAGGAGTAG AAAGAATCGT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       1260       1270       1280       1290       1300
B392            TGAATCTGGG ATGAATAATA TATTTAGAGC TGGTGGCGCA CATGATGCAA
186             TGAATCTGGG ATGAATAATA TATTTAGAGC TGGTGGCGCA CATGATGCAA
385             TGAATCTGGG ATGAATAATA TATTTAGAGC TGGTGGCGCA CATGATGCAA
443             TGAATCTGGG ATGAATAATA TATTTAGAGC TGGTGGCGCA CATGATGCAA
500             TGAATCTGGG ATGAATAATA TATTTAGAGC TGGTGGCGCA CATGATGCAA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       1310       1320       1330       1340       1350
B392            TGCGCCCTCT TCAACGTTTA GTTCGATTTG TTTCTCATGA AAGACCATAA
186             TGCGCCCTCT TCAACGTTTA GTTCGATTTG TTTCTCATGA AAGACCATAT
385             TGCGTCCACT TCAACGTTTA GTTCGATTTG TTTCTCATGA AAGACCATAT
443             TGCGTCCACT TCAACGTTTA GTTCGATTTG TTTCTCATGA AAGACCATAT
500             TGCGCCCACT TCAACGTTTA GTTCGATTTG TTTCTCATGA AAGACCATAT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       1360       1370       1380       1390       1400
B392            TAACTCACCA CTAAGGATGT ATCTGTCGAA ATAGAGCAAA CCCGCTTTCT
186             AACTTCACCA CTAAGGATGT ATCTGTCGAA ATAGAGCAAA CCCGCTTTCT
385             AACTTCACCA CTAAGGATGT ATCTGTCGAA ATAGAGCAAA CCCGCTTTCT
443             AACTTCACCA CTAAGGATGT ATCTGTCGAA ATAGAGCAAA CCCGCTTTCT
500             AACTTCACCA CTAAGGATGT ATCTGTCGAA ATAGAGCAAA CCCGCTTTCT

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                       1410       1420       1430
B392            TGAAGAAGAT AAATTCTTGG TTTTCGTCCC T[TAA]
186             TGAAGAAGAT AAATTCTTGG TTTTCGTCCC T[TAA]
385             TGAAGAAGAT AAATTCTTGG TTTTCGTCCC T[TAA]
443             TGAAGAAGAT AAATTCTTGG TTTTCGTCCC T[TAA]
500             TGAAGAAGAT AAATTCTTGG TTTTCGTCCC T[TAA]
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Appendix C.

luxA consensus sequence

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                ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
B392            ATGAAATTTG GAAACTTCCT TCTCACTTAT CAGCCACCTG AGCTATCTCA
186             ATGAAATTTG GAAACTTCCT TCTCACTTAT CAGCCACCTG AGCTATCTCA
385             ATGAAATTTG GAAACTTCCT TCTCACTTAT CAGCCACCTG AGTTATCTCA
443             ATGAAATTTG GAAACTTCCT TCTCACTTAT CAGCCACCTG AGCTATCTCA
500             ATGAAATTTG GAAACTTCCT TCTCACGTAT CAGCCACCTG AGCTATCTCA

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                        60         70         80         90        100
B392            GACCGAAGTG ATGAAGCGAT TGGTTAATCT GGGCAAAGCG TCTGAAGGTT
186             GACCGAAGTG ATGAAGCGAT TGGTCAATCT GGGCAAAGCG TCTGAAGGTT
385             GACCGAAGTG ATGAAGCGAT TGGTTAATCT GGGCAAAGCG TCTGAAGGTT
443             GACCGAAGTG ATGAAGCGAT TGGTTAATCT GGGCTAAGCG TCTGAAGGTT
500             GACCGAAGTG ATGAAGCGAT TGGTTAATCT GGGCAAAGCG TCTGAAGGTT

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                       110        120        130        140        150
B392            GTGGCTTCGA CACCGTTTGG TTGCTAGAGC ACCACTTCAC TGAATTTGGG
186             GTGGCTTCGA CACCGTTTGG TTGCTAGAGC ACCACTTCAC TGAATTTGGG
385             GTGGTTTCGA CACTGTTTGG TTACTAGAGC ACCACTTCAC TGAATTTGGG
443             GTGGTTTCGA CACTGTTTGG TTACTAGAGC ACCACTTCAC TGAATTTGGG
500             GTGGCTTCGA CACAGTTTGG TTGCTAGAGC ACCACTTCAC TGAATTTGGG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       160        170        180        190        200
B392            TTGTTAGGGA ATCCTTATGT TGCTGCCGCA CACCTATTAG GTGCGACAGA
186             TTGTTAGGGA ATCCTTATGT TGCTGCCGCA CACCTATTAG GTGCGACAGA
385             TTGTTAGGGA ATCCTTATGT TGCTGCCGCA CATCTATTAG GTGCGACAGA
443             TTGTTAGGGA ATCCTTATGT TGCTGCCGCA CATCTATTAG GTGCGACAGA
500             TTGCTAGGGA ATCCTTATGT TGCTGCCGCA CACCTATTAG GTGCGACAGA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       210        220        230        240        250
B392            AACGCTCAAC GTTGGCACTG CAGCTATCGT ATTGCCGACT GCCCATCCGG
186             AACGCTCAAC GTTGGCACTG CAGCTATCGT ATTGCCGACT GCCCATCCGG
385             AAAGCTCAAT GTTGGCACCG CAGCTATTGT ATTGCCGACT GCCCATCCGG
443             AAAGCTCAAT GTTGGCACCG CAGCTATTGT ATTGCCGACC GCCCATCCGG
500             AACGCTCAAC GTTGGCACTG CAGCTATCGT ATTGCCGACT GCCCATCCAG
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       260        270        280        290        300
B392            TTCGACAAGC AGAAGACGTA AACCTACTGG ATCAAATGTC AAAAGGACGA
186             TTCGACAAGC AGAAGACGTA AACCTACTGG ATCAAATGTC AAAAGGACGA
385             TTCGACAAGC AGAAGACGTA AACCTACTGG ATCAAATGTC AAAAGGACGA
443             TTCGACAAGC AGAAGACGTA AACCTACTGG ATCAAATGTC AAAAGGACGA
500             TTCGACAAGC AGAAGACGTA AACCTACTGG ATCAAATGTC GAAAGGACGA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       310        320        330        340        350
B392            TTCCGTTTTG GTATTTGTCG CGGTTTGTAC GATAAAGATT TTCGTGTCTT
186             TTCCGTTTTG GTATTTGTCG CGGTTTGTAC GATAAAGATT TTCGTGTCTT
385             TTCCGTTTTG GTATTTGTCG CGGTTTGTAC GACAAAGATT TCCGTGTCTT
443             TTCCGTTTTG GTATTTGTCG CGGTTTGTAC GACAAAGATT TCCGTGTCTT
500             TTCCGTTTTG GTATTTGTCG CGGTTTGTAC GACAAAGATT TCCGTGTCTT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       360        370        380        390        400
B392            TGGTACAGAC ATGGATAACA GCCGAGCCTT AATGGACTGT TGGTATGACT
186             TGGTACAGAC ATGGATAACA GCCGAGCCTT AATGGACTGT TGGTATGACT
385             TGGTACAGAC ATGGATAACA GCCGAGCCTT AATGGACTGT TGGTATGACT
443             TGGTACAGAC ATGGATAACA GCCGAGCCTT AATGGACTGT TGGTATGACT
500             TGGTACAGAC ATGGATAACA GCCGAGCCTT AATGGACTGT TGGTATGACT

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                       410        420        430        440        450
B392            TGATGAAAGA AGGCTTCAAT GAAGGCTATA TCGCGGCGGA TAACGAACAT
186             TGATGAAAGA AGGCTTCAAT GAAGGCTATA TCGCGGCGGA TAACGAACAT
385             TGATGAAAGA AGGCTTCAAT GAAGGCTATA TCGCGGCGGA TAACGAACAT
443             TGATGAAAGA AGGCTTCAAT GAAGGCTATA TCGCGGCGGA TAACGAACAT
500             TGATGAAAGA AGGCTTCAAT GAAGGCTATA TCGCGGCGGA TAACGAACAT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       460        470        480        490        500
B392            ATTAAGTTCC CGAAAATCCA ACTGAATCCA TCGGCTTACA CACAAGGTGG
186             ATTAAGTTCC CGAAAATCCA ACTGAATCCA TCGGCTTACA CACAAGGTGG
385             ATTAAGTTCC CGAAAATCCA ACTGAATCCA TCGGCTTACA CACAAGGTGG
443             ATTAAGTTCC CGAAAATCCA ACTGAATCCA TCGGCTTACA CACAAGGTGG
500             ATCAAGTTCC CGAAAATCCA ACTGAATCCA TCGGCTTACA CACAAGGTGG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       510        520        530        540        550
B392            TGCTCCTGTT TATGTCGTCG CGGAGTCAGC ATCAACGACA GAATGGGCTG
186             TGCTCCTGTT TATGTCGTCG CGGAGTCAGC ATCAACGACA GAATGGGCTG
385             CGCTCCTGTT TATGTCGTCG CGGAGTCAGC GTCAACGACA GAATGGGCTG
443             CGCTCCTGTT TATGTCGTCG CGGAGTCAGC ATCAACGACA GAATGGGCTG
500             CGCGCCTGTT TATGTAGTCG CGGAGTCAGC ATCAACGACA GAATGGGCTG
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       560        570        580        590        600
B392            CAGAGCGTGG CCTACCAATG ATTCTAAGCT GGATCATCAA CACTCACGAG
186             CAGAGCGTGG CCTACCAATG ATTCTAAGCT GGATCATCAA CACTCACGAG
385             CAGAGCGTGG CCTACCAATG ATTCTAAGCT GGATCATCAA CACTCACGAG
443             CAGAGCGTGG CCTACCAATG ATTCTAAGCT GGATCATCAA CACTCACGAG
500             CAGAGCGTGG CCTACCAATG ATTCTAAGCT GGATCATCAA CACTCACGAG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       610        620        630        640        650
B392            AAGAAAGCGC AGCTTGATCT TTACAACGAA GTCGCGACTG AACATGGCTA
186             AAGAAAGCGC AGCTTGATCT TTACAACGAA GTCGCGACTG AACATGGCTA
385             AAGAAAGCGC AGCTTGATCT TTACAATGAA GTTGCGACTG AACATGGCTA
443             AAGAAAGCGC AGCTTGATCT TTACAATGAA GTCGCGACTG AGCATGGCTA
500             AAGAAAGCGC AGCTTGATCT TTACAATGAA GTCGCGACTG AACATGGCTA

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                       660        670        680        690        700
B392            CGATGTGACT AAGATTGACC ACTGTTTGTC TTACATCACC TCCGTCGATC
186             CGATGTGACT AAGATTGACC ACTGTTTGTC TTACATCACC TCCGTCGATC
385             CGATGTGACT AAGATTGACC ACTGTTTGTC TTACATCACC TCCGTCGATC
443             CGATGTGACT AAGATTGACC ACTGTTTGTC TTACATCACC TCCGTCGATC
500             CGATGTGACT AAGATTGACC ACTGTTTGTC TTACATCACC TCCGTCGATC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       710        720        730        740        750
B392            ATGACTCAAA TAGAGCCAAA GATATTTGCC GCAACTTCTT GGGCCATTGG
186             ATGACTCAAA TAGAGCCAAA GATATTTGCC GCAACTTCTT GGGCCATTGG
385             ATGACTCAAA TAAAGCCAAA GATATTTGCC GCAACTTCTT GGGTCATTGG
443             ATGACTCAAA TAAAGCCAAA GATATTTGCC GCAACTTCTT GGGTCATTGG
500             ATGACTCAAA TAAAGCCAAA GATATTTGCC GCAACTTCTT GGGTCATTGG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       760        770        780        790        800
B392            TACGACTCAT ACGTGAATGC CACCAAGATT TTTGACGACT CTGACCAAAC
186             TACGACTCAT ACGTGAATGC CACCAAGATT TTTGACGACT CTGACCAAAC
385             TACGACTCAT ACGTGAATGC CACAAAGATT TTTGACGACT CTGACCAAAC
443             TACGACTCAT ACGTGAATGC CACCAAGATT TTTGACGACT CTGACCAAAC
500             TACGACTCAT ACGTGAATGC CACCAAGATT TTTGACGACT CTGACCAAAC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       810        820        830        840        850
B392            AAAAGGTTAC GACTTCAATA AAGGTCAATG GCGTGATTTT GTGTTGAAAG
186             AAAAGGTTAC GACTTCAATA AAGGTCAATG GCGTGATTTT GTGTTGAAAG
385             AAAAGGCTAC GACTTCAATA AAGGTCAATG GCGTGATTTT GTCTTGAAAG
443             AAAAGGCTAC GACTTCAATA AAGGCCAATG GCGCGATTTT GTCTTGAAAG
500             AAAAGGCTAC GACTTCAATA AAGGTCAATG GCGTGATTTT GTGTTGAAAG
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       860        870        880        890        900
B392            GCCACAAAGA CACCAATCGC CGAATTGATT ACAGCTACGA AATCAACCCA
186             GCCACAAAGA CACCAATCGC CGAATTGATT ACAGCTACGA AATCAACCCA
385             GCCACAAAGA CACCAATCGC CGAATTGATT ACAGCTACGA AATCAACCCA
443             GCCACAAAGA CACCAACCGC CGAATTGATT ATAGCTACGA AATCAACCCA
500             GCCACAAAGA CACTAATCGC CGAATTGATT ACAGCTACGA AATCAACCCA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       910        920        930        940        950
B392            GTAGGGACGC CTGAAGAGTG TATCGCGATT ATCCAGCAAG ATATTGATGC
186             GTAGGGACGC CTGAAGAGTG TATCGCGATT ATCCAGCAAG ATATCGATGC
385             GTAGGGACGC CTGAAGAGTG TATCGCGATT ATCCAGCAAG ATATCGATGC
443             GTAGGGACGC CTGAAGAGTG TATCGCGATT ATCCAGCAAG ATATCGATGC
500             GTAGGGACGC CTGAAGAGTG TATCGCGATT ATCCAGCAAG ATATCGATGC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       960        970        980        990       1000
B392            GACGGGTATT GACAATATTT GTTGTGGTTT TGAAGCAAAC GGTTCTGAAG
186             GACGGGTATT GACAATATTT GTTGTGGTTT TGAAGCAAAC GGTTCTGAAG
385             GACGGGTATT AACAATATTT GTTGTGGTTT TGAAGCAAAC GGTTCTGAAG
443             GACGGGTATT GACAATATTT GTTGTGGTTT TGAAGCAAAC GGTTCTGAAG
500             GACGGGTATT AACAATATTT GTTGTGGTTT TGAAGCAAAC GGTTCTGAAG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                      1010       1020       1030       1040       1050
B392            AAGAAATTAT CGCATCTATG AAGCTATTCC AGTCTGATGT GATGCCATAT
186             AAGAAATTAT CGCATCTATG AAGCTATTCC AGTCTGATGT GATGCCATAT
385             AAGAAATTAT CGCATCTATG AAGCTATTCC AGTCTGATGT GATGCCATAT
443             AAGAAATTAT CGCATCTATG AAGCTATTCC AGTCCGATGT GATGCCATAT
500             AAGAAATTAT CGCATCTATG AAGCTATTCC AGTCTGATGT GATGCCATAT

                ....|....| ....|...
                      1060
B392            CTCAAAGAAA AACAG<u>**TAA**</u>
186             CTCAAAGAAA AACAG<u>**TAA**</u>
385             CTCAAAGAAA AACAG<u>**TAA**</u>
443             CTCAAAGAAA AACAG<u>**TAA**</u>
500             CTCAAAGAAA AACAG<u>**TAA**</u>
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